REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	04-11-96	FINAL 01 Dec 92 to 30 Nov 95

4. TITLE AND SUBTITLE
LONG-TERM POTENTIATION: ROLE OF HIPPOCAMPAL ADRENAL STEROID TYPE I & TYPE II RECEPTORS IN THE DIURNAL RHYTHM & THE IMPACT OF STRESS

5. FUNDING NUMBERS
F49620-94-1-0048 P00003
93
2312/BS
61102F

6. AUTHOR(S)
Dr. Bruce S. McEwen
Dr. Ana Maria Magarinos
Dr. Constantine Pavlides

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Rockefeller University
1230 York Avenue
New York, N.Y. 10021

8. PERFORMING ORGANIZATION REPORT NUMBER
ATR-3

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
Building 410
Bolling AFB DC 20332-6448
Dr Hallad

AFOSR-TR-96
0210

11. SUPPLEMENTARY NOTES

DISTRIBUTION STATEMENT A
Approved for public release
Distribution Unlimited

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited.

12b. DISTRIBUTION CODE

19960520 071

13. ABSTRACT (Maximum 200 words)

It was determined that adrenal steroids modulated long-term potentiation (LTP) in the hippocampus through activation of Type I (mineralocorticoid) and Type II (glucocorticoid) adrenal steroid receptors: Type I receptors enhanced while Type II receptors suppressed LTP and produced long-term depression (LTD). These effects were seen in the dentate gyrus (DG) of anesthetized and freely behaving rats and in the CA1 hippocampal field, in vitro. Within the CA3 hippocampal field, adrenal steroids modualted LTP for the commissural/associational but not the mossy fiber input. It was also observed that the immediate early gene c-fos was not induced by LTP while zif/268 was. Induction of zif/268 at initial site of LTP was mainly seen for the aldosterone-treated group, but more importantly, there was also a delayed and bilateral activation of zif/268 at 24h in subfields of the hippocampus downstream to the potentiated cell group (e.g., the CA3 and CA1 fields, only in the aldosterone-treated animals. This activation indicated sequential processing of information over time and may reflect the prolongation in LTP produced by aldosterone. GAP-43, a gene related to synaptogenesis, was not activated by LTP, but was modulated by the adrenal steroid treatments.

14. SUBJECT TERMS
Hippocampus, adrenal steroids, receptors, long-term potentiation, learning, jet lag, immediate early genes

15. NUMBER OF PAGES

16. PRICE CODE

DTIC QUALITY INSPECTED 1

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	SAR

Objectives

Adrenal steroids are secreted during stress, but also show diurnal rhythmicity during the sleep-wake cycle. It has become evident, in the past several years, that adrenal steroids can affect brain function and in particular cognitive processes. Thus, both stress or shifts of the diurnal cycle, which can occur with shifts in work schedule or jet lag, commonly experienced by military personnel in general and aviators and air crews in particular, can have major consequences for human performance. To provide a basis for understanding environmental control of human performance, the main goal of this project was to investigate effects of adrenal steroids on synaptic plasticity in the hippocampus, a brain structure which contains the highest concentration of adrenal steroid receptors, and which is closely associated with episodic and spatial learning and memory. The model of neuronal plasticity used to investigate these processes was LTP and LTD, the majority of the studies were performed *in vivo*. Furthermore, the molecular mechanisms that may accompany the physiological changes were also investigated.

Accomplishments

Adrenal steroid effects on hippocampal LTP.

In an initial investigation (10) we showed, in the DG of anesthetized animals, that an acute injection of corticosterone that produced high blood levels of hormone (such as may occur with a moderate level of stress), produced a suppression in synaptic plasticity (see Fig. below).

In the same study, a similar suppression in synaptic plasticity was also observed with chronic (21d) administration of corticosterone. These effects were observed 48h following the last corticosterone injections, at basal plasma corticosterone levels. We hypothesized these effects to be subserved by different mechanisms - the acute effects resulting from direct actions of corticosterone on the genome while the chronic effects resulting from neuroanatomical changes that we have shown to occur in the hippocampus after daily (21d) administration of corticosterone or daily (21d) restraint stress.

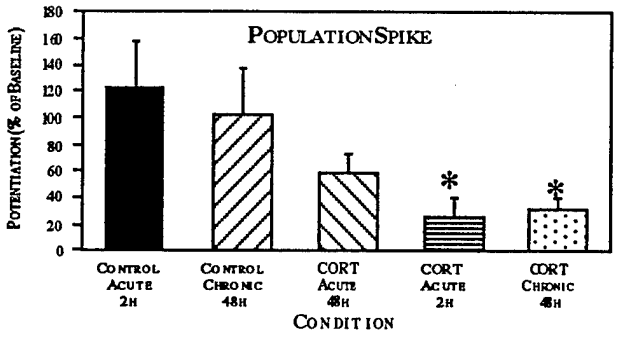

Fig. 1. Averaged LTP results for control and corticosterone-treated animals. The degree of LTP in the acute-CORT-48h (n=8) animals was significantly lower (*P<0.05) than control animals (i.e., chronic-control-48h (n=5) and acute-control-2h and 48h (n=4), were combined since they did not differ from each other). Although lower than the control groups, LTP for the acute-CORT-48h animals (n=10) was not significantly different.

Biphasic effects of adrenal steroids on LTP.

In a subsequent study (9) we showed (again in the DG of anesthetized animals) that adrenal steroids had a biphasic effect, in that high levels of corticosterone, which activate Type II receptors produced a suppression of LTP while activation of Type I receptors produced an enhancement in LTP (see Fig. below). This is of considerable significance in that it shows that peak levels of cognitive function could occur when an animal or human is alert or mildly stressed while higher levels of stress are detrimental to cognitive performance.

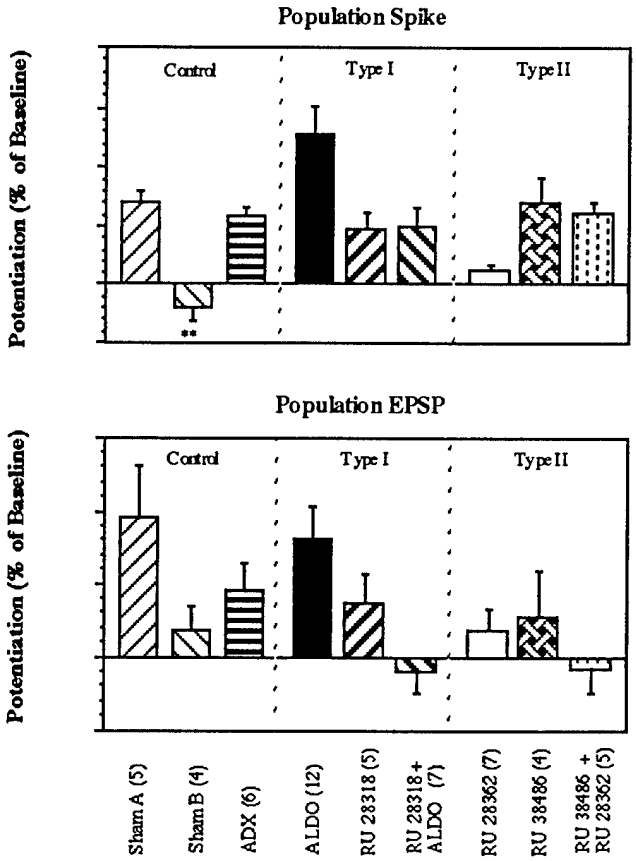

Fig. 2. Group results of high frequency stimulation on population spike (A) and EPSP slope (B). Bar graphs represent mean (±SEM) potentiation for each of the groups of animals included in the experiment. In comparison to ADX controls, the Type I adrenal steroid agonist, ALDO, produced a significant enhancement in LTP as measured by the population spike. This enhancement was reversed by the Type I antagonist RU 28318. In contrast, the Type II agonist RU 28362 produced a significant reduction which was reversible with a preadministration of the Type II antagonist RU 38486. With respect to the slope of the EPSP, none of the Type I or Type II agonist/antagonist groups were significantly different than the ADX group, although a trend similar to the spike was observed. The numbers in parenthesis represent the number of animals in each group. (** $P < 0.001$; * $P < 0.05$). The sham-operated animals were split into two groups based on post-hoc analysis of plasma CORT levels and LTP results. Animals in the Sham A group had levels lower than 45μg/dl and showed 'normal' LTP while those in the Sham B group had plasma CORT values higher than this and showed either suppressed LTP or in some cases long-term depression.

These findings were replicated and extended in another experiment (3), performed in the DG of freely behaving animals (see Fig. below). In this study we showed that aldosterone not only enhanced synaptic plasticity, but also prolonged its maintenance. This study was also of significance since anesthetics can have profound effects, in and of themselves.

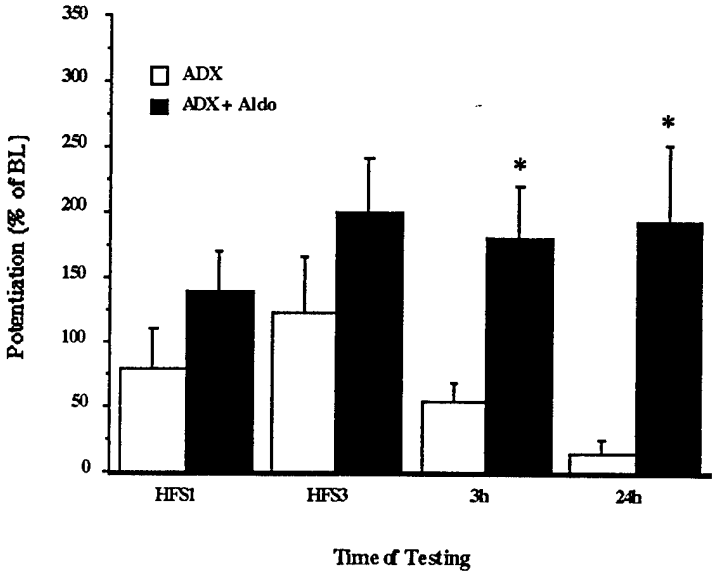

Fig. 3. Averaged results showing ALDO effects on LTP in the dentate gyrus. Following baseline recording, a set of three HFS were applied and changes in the size of the population spike were determined, in comparison to baseline. The first HFS produced significant LTP for both the ADX+ALDO and ADX+vehicle animals, with higher increases seen after the third HFS. Although the ADX+ALDO show somewhat higher LTP than the ADX+vehicle animals, this difference was not significant. Thus, comparable LTP was obtained for both groups. However, while LTP decayed substantially by 3 hours and returned to baseline levels by 24h in the ADX+vehicle group, it persisted in the ADX+ALDO animals not only at three but also at 24h following HFS. The asterisk indicates significant ($p < 0.05$) differences between ADX+ALDO vs ADX+vehicle animals at the times indicated.

Long-term depression.

In the course of these experiments, we noticed that with very high levels of glucocorticoids (as may occur under severe stress), electrical stimulation which normally produces an enhancement, produced LTD of synaptic transmission, meaning that information flow through the hippocampus would be blocked. In the next experiment (4) we substantiated these early observations, in the DG of anesthetized animals (see Fig. below). This finding is of major significance in that it may explain deficits in memory or perhaps complete lapses in memory associated with severe stress.

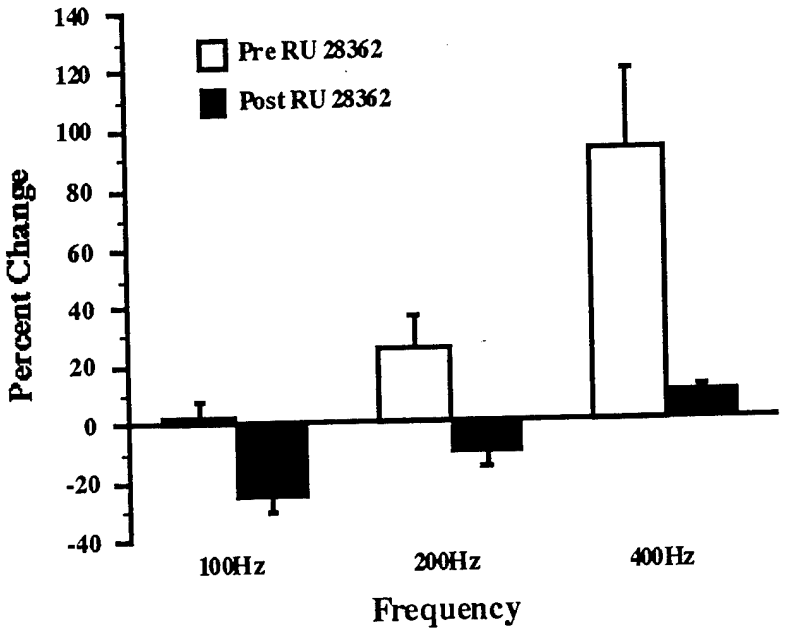

Fig. 4. Representative examples of the effects of RU 28362 on hippocampal plasticity. In both A and B, following stable baseline recording, a number of high frequency stimuli were applied and in each case recording continued. RU 28362 was then administered and approximately 30-60min later HFS was again applied followed by field potential recording. As becomes evident in both examples, HFS at 200Hz and 400Hz prior to the RU 28362 produced significant LTP. In contrast, similar HFS following the RU 28362 injection had the opposite effect, i.e., a reduction or long term depression of the field potentials. Administration of RU 28362 by itself did not produce noticeable changes with the low frequency stimulation. Changes are expressed as percent from previous condition.

Adrenal steroid effects on LTP in the hippocampal CA1 field.

A correlation between an elevation of adrenal steroids with stress and learning and memory processes had previously been demonstrated. Further, other workers found that painful shock stress produces a deficit in LTP in the CA1 hippocampal field of an in vitro slice preparation via a mechanism involving opioid peptides. Our next experiment addressed the question of whether or not adrenal steroids could also produce deficits in LTP in the CA1 field of hippocampal slices. This experiment is now completed and a manuscript has been submitted for publication (6). Briefly, the results confirmed our findings in the DG in the *in vivo* preparation; namely, that Type I adrenal steroid receptors enhanced synaptic plasticity while Type II receptors suppressed it (see Fig. below). This experiment is of further significance, however, in that a slice preparation was established in our laboratory which will allow us to further investigate the neuronal mechanisms underlying the changes in synaptic plasticity, which cannot be studied, *in vivo*. These experiments are of great importance since they will provide considerable insight into the neuronal mechanisms underlying the steroid effects on synaptic plasticity.

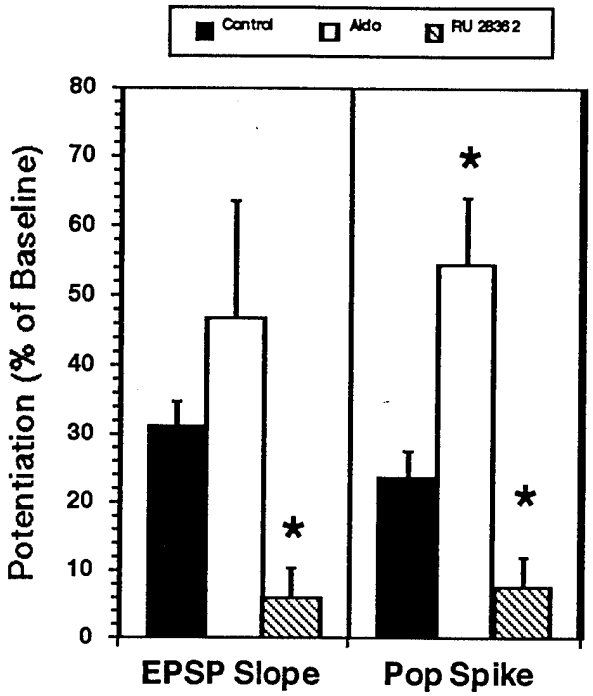

Fig. 5. Averaged results of the effects of MR and GR activation on long-term potentiation. Measurements were made of the slope of the fEPSP and the population spike. High frequency stimulation in the slices from control injected animals (ADX + non-ADX control, n = 9) produced a moderate degree of potentiation (in comparison to baseline), as measured both for the fEPSP slope (31.0% ±6.1%SEM) and the population spike (23.5% ±4.3%SEM). In comparison, similar stimulation in the slices from animals injected with aldosterone (n = 7) produced higher (approaching significance, p = 0.16) potentiation for the EPSP slope (46.7% ±16.9%SEM) and significantly higher potentiation for the population spike (54.6% ±9.3%SEM). In contrast, high-frequency stimulation in the slices from the RU 28362 injected animals (n = 7) produced almost complete suppression in LTP as measured both for the population slope (5.9% ±4.5%SEM) and the population spike (7.4% ±4.6%SEM). This potentiation was significantly lower ($p < 0.05$) than the potentiation from control slices.

Adrenal steroid effects on LTP in the CA3 hippocampal field.

From other work in our laboratory, we have shown that chronic stress (21d) produces a reduction in the length and branching of apical dendrites in hippocampal CA3c neurons. These effects appear to be dependent on glucocorticoids as well as excitatory amino acids acting via NMDA receptors. As stated in the original proposal, we wanted to concentrate our electrophysiological investigations in this area. We have already started these experiments and have made significant progress. The objective thus far has been to record in the cell body layer, in an initial attempt at replicating the adrenal steroid effects in CA3. Stimulating electrodes were placed ipsilaterally in the mossy fibers and in the contralateral hemisphere for stimulating the commissural/associational pathway. A stimulating electrode was also placed in the perforant pathway to record in the dentate gyrus. This allowed us to replicate within the same animal our previous findings of adrenal steroid effects in the dentate gyrus. It is well known that LTP in the commissural/associational and perforant pathway is N-methyl-D-aspartate (NMDA) dependent

while LTP in the mossy fiber pathway is not NMDA but rather opioid peptide dependent. Testing from all three pathways simultaneously allowed us to determine if adrenal steroid effects on LTP may be mediated via the NMDA receptor.

Unlike the ease of recording and stimulation in the DG and CA1 field, however, getting the precise coordinates that would produce good field potentials from both stimulation sites proved to be more difficult than anticipated. Furthermore, we were faced with the problem of choosing the appropriate stimulation coordinates for inducing an intermediate level of LTP which would allow us to determine the effects of adrenal steroids on plasticity. In pilot studies we attempted to use stimulation parameters previously used in the DG and CA1 field, however, these proved to be ineffective in inducing LTP in this field. We have since determined the correct stimulation parameters and are well underway in completing the study in the CA3 field. The results, thus far, are extremely interesting (see fig. below). Similar to what was observed in the DG and CA1, for the commissural/associational pathway activation of Type I receptors produced a facilitation while activation of Type II receptors produced a suppression in LTP. This was not the case for the mossy fiber input for which adrenal steroids did not modulate synaptic plasticity. These results are of extreme significance in that they substantiate our previous hypothesis that the deleterious affects of adrenal steroids on the morphological degeneration in the CA3 field may be mediated via activation of the NMDA receptor.

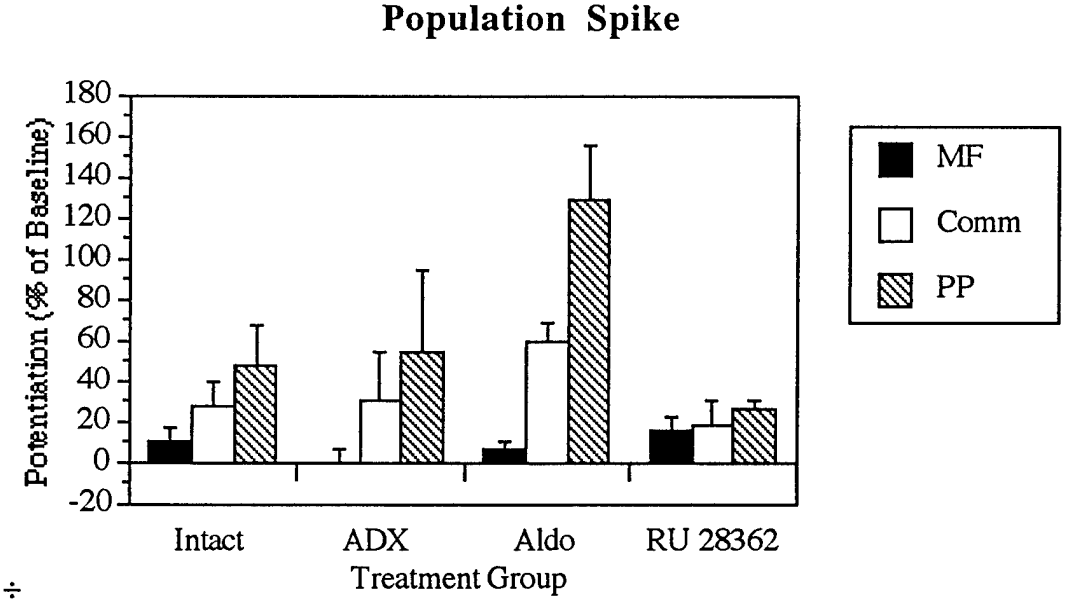

Fig. 6. Effects of adrenal steroid, Type I and Type II receptors on LTP in the mossy fiber, commissural and perforant pathway inputs. Activation of Type I receptors produced a significant increase in LTP for the commissural/associational and perforant pathway. Conversely, activation of Type II receptors produced a suppression in LTP for these inputs. On the other hand, adrenal steroids did not have a significant effect on LTP for the mossy fiber pathway.

As mentioned above, adrenal steroids affect mainly the apical but not the basal dendrites of the CA3 hippocampal field. Thus, the functional changes that may result from these could be rather complex and may not be easily determined or obvious by limiting our recording to the pyramidal cell layer. For this reason we have set up to determine effects of adrenal steroids on LTP, using current source density analysis which allows the detection of changes in currents and sinks throughout the dendritic field. This involves using a moveable microelectrode and recording evoked potentials at 50μm intervals, stemming the width of the CA3 layer. Using this technique will also allow us to determine changes in physiology and plasticity resulting from chronic stress, which, judging from our neuroanatomical data, should be confined to the basal dendrites of the CA3c field. These experiments have been started recently, therefore, the results are still very preliminary to determine the effects of stress.

Effects of adrenal steroids on immediate early genes.

As stated above, we have thus far determined that c-fos is not induced with LTP while zif/268 is. This is turning out to be a rather interesting story. In preliminary studies, thus far, we have observed that high-frequency stimulation to one hemisphere produced unilateral zif/268 gene expression at the initial site of potentiation; i.e., the DG, mainly for the aldosterone-treated group and only at the 2h test interval. More interestingly, at the 24h interval, there was a <u>delayed</u> expression of zif/268 at the CA1 hippocampal field for the aldosterone-treated group. At this interval, reactivation was bilateral (see Fig. A and B, below). A possible interpretation of this result is that the enhanced activation and prolongation of LTP by aldosterone in one hippocampal subfield allows for the transfer of information to other parts of the hippocampus and perhaps the rest of the brain at later time intervals. This possibility will have to be further investigated, but if true it will be an extremely exciting result.

We have also found that GAP-43, a gene related to synaptogenesis, is not activated by LTP, but is modulated by the adrenal steroid treatments (see Fig. C and D, below).

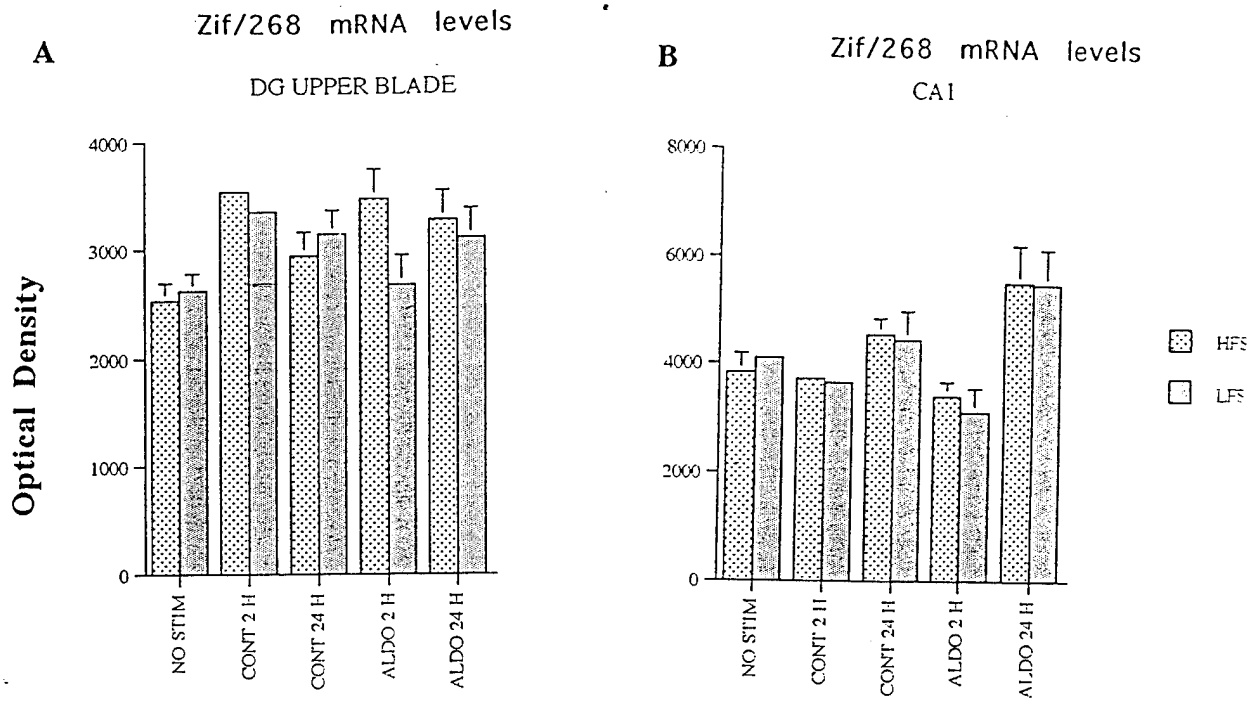

Fig. 7. Effects of unilateral high-frequency stimulation to the perforant pathway on zif/268 expression in the hippocampus at 2h or 24h following the stimulation. High-frequency stimulation produced zif/268 expression at the potentiated, ipsilateral, ipsilateral DG mainly for the aldosterone injected animals (A). For this group, zif/268 mRNA was again seen at the CA1 hippocampal field at the 24h interval (B).

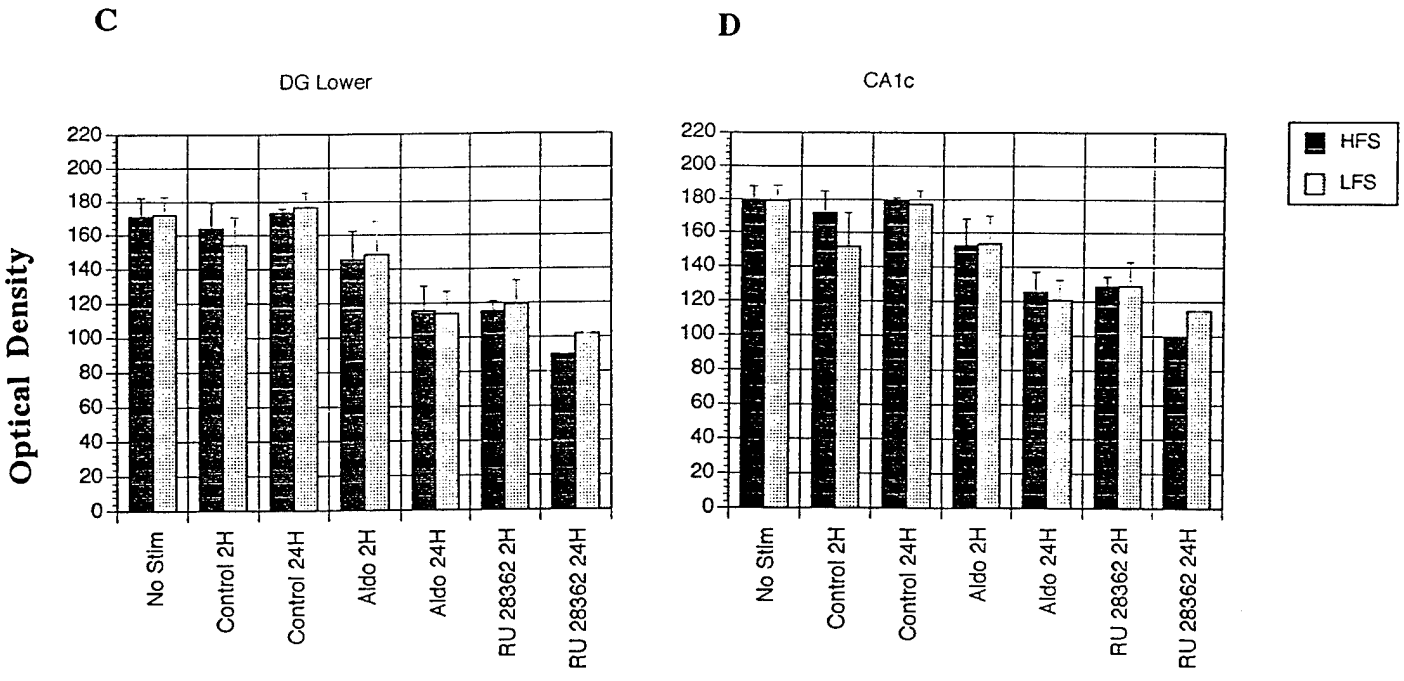

Fig. 8. GAP-43 expression in hippocampi of animals treated with aldosterone or RU 28362, tetanized in the perforant pathway unilaterally and tested for LTP at either 2h or 24h following LTP induction. LTP did not affect GAP-43 expression, for any of the groups tested or at any test interval. However, both aldosterone and RU 28362 produced a suppression in GAP-43 mRNA at the 24h test interval. (Similar results were obtained for all of the hippocampal fields tested.)

Now that the basic foundation has been laid down regarding the role of adrenal steroid Type I and Type II receptors in modulating excitability and LTP in the hippocampus, it will be possible in the future to address the issue of diurnal variations in cognitive function and the effects of stress. Recent work at Hunter College and Rockefeller University by Dr. Paul Vaher and Dr. Victoria Luine has revealed that Type I receptor activation, in adrenalectomized rats, improves learning/memory in a spatial task (8-arm radial maze) and that Type II receptor stimulation impairs performance. These are exactly the effects that would be predicted from the LTP results obtained in the present grant. Moreover, there is a growing body of neuropsychological data on humans that suggests that adrenal steroids produce biphasic effects on cognitive function related to the hippocampus, with moderate levels of adrenal steroids facilitating cognition and high levels inhibiting it.

Personnel Supported:
Dr. Constantine Pavlides
Dr. Ana-Maria Magariños

Publications:

1. McEwen, B. S., Albeck, D., Cameron, H., Chao, H. M., Gould, E., Hastings, N., Kuroda, Y., Magariños, A. M., McKittrick, C. R., Orchinik, M., Pavlides, C. and Watanabe, Y. 1995 Stress and the brain: a paradoxical role for adrenal steroids. In Vitamins and Hormones Eds. G. Litwack 51 pp. 371-402. Academic Press, San Diego.

2. McEwen, B. S., Cameron, H., Chao, H. M., Gould, E., Luine, V., Magariños, A. M., Pavlides, C., Spencer, R. L., Watanabe, Y. and Woolley, C. 1993 Resolving a mystery: progress in understanding the function of adrenal steroid receptors in hippocampus. In Neuroscience: From the Molecular to the Cognitive Eds. F. E. Bloom 100 pp. 149-155. Elsevier, New York.

3. Pavlides, C., Kimura, A., Magariños, A. M. and McEwen, B. S. (1994) Type I adrenal steroid receptors prolong hippocampal long-term potentiation. *Neuroreport*, **5**, 2673-2677.

4. Pavlides, C., Kimura, A., Magariños, A. M. and McEwen, B. S. (1995) Hippocampal homosynaptic long-term depression/depotentiation induced by adrenal steroids. *Neuroscience*, **68**, 379-385.

5. Pavlides, C., Magariños, A., Gould, E., Hsu, M., Pierre, E., Watanabe, Y., McEwen, B. S. and Buzsáki, G. (1993) Adrenalectomy-induced degeneration of granule cells but not CA3 interneurons is prevented by corticosterone treatment. *Soc Neurosci Abstr*,

6. Pavlides, C., Ogawa, S., Kimura, A. and McEwen, B. S. (1995) Adrenal steroid effects on long-term potentiation in the CA1 field of hippocampal slices. *Soc Neurosci Abstr*, **25**,

7. Pavlides, C., Ogawa, S., Kimura, A. and McEwen, B. S. (1996) Role of adrenal steroid mineralocorticoid and glucocorticoid receptors in long-term potentiation in the CA1 field of hippocampal slices. *Submitted*,

8. Pavlides, C., Watanabe, Y., Magariños, A. M. and McEwen, B. S. (1992) Effects of glucocorticoid Type I and Type II agonists on hippocampal long-term potentiation. *Soc Neurosci Abstr*, **18**, 343.

9. Pavlides, C., Watanabe, Y., Magariños, A. M. and McEwen, B. S. (1995) Opposing roles of Type I and Type II adrenal steroid receptors in hippocampal long-term potentiation. *Neuroscience*, **68**, 387-394.

*10. Pavlides, C., Watanabe, Y. and McEwen, B. S. (1993) Effects of glucocorticoids on hippocampal long-term potentiation. *Hippocampus*, **3**, 183-192.

* This publication culminated from support from a previous grant.